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Chapter 6

ACTION MODE OF TRIAZINES AND TOXIC EFFECTS ON VERTEBRATES

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ABSTRACT

Triazines are the family of herbicides that include atrazine, ametryn and simazine that are widely used in Brazil and can contaminate groundwater. Cattle can accumulate herbicides in their body through ingestion plants infested with these compounds and one of the ways, by which, human beings are exposed to atrazine is through cattle meat and milk consumption. The toxicity of these compounds can be explained mainly by their interaction with microsomal biotransformation processes. The herbicides per se or their metabolites or the secondary products of oxidative stress interact with biomolecules such proteins and DNA affecting a lot of cellular types. Cellular effects of chemicals might involve recruitment or de-repression of cell death mechanisms. Whether a cell survives or dies in the presence of a chemical insult is often determined by proliferative status, repair enzyme capacity, and the ability to induce proteins that either promote or inhibit the cell death process. In this chapter we will present an extensive bibliographical review about this herbicide class focusing its effects on vertebrates, looking for defense cellular mechanisms, at morphological and biochemical levels.

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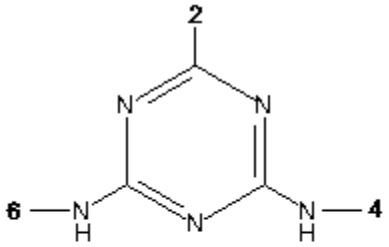
INTRODUCTION
TRIAZINE HERBICIDES – ABSORPTION AND BIOTRANSFORMATION

Triazine herbicides are used to control broad weeds and grasses at crops with great economic importance. Their herbicide action mechanism is mainly based on inhibition of Photosystem II (PSII) of chloroplast electron chain [1].

Up to 380,000 libs of ametryn active ingredient are used per year. Sixty per cent of the annual use of ametryn is used with corn, 20% with pineapple and 20% with sugarcane. 76.4 million pounds of Atrazine was used, only in USA, per year, 86% on corn, 10% on sorghum and 10% on sugar cane crops. Simazine is used on artichokes, asparagus, beans and other deep-rooted crops [2].

Technical grade ametryn (95%) pure is slightly soluble in water (18.5 mg/L) and presents an octanol/water partition coefficient of 676 at pH 7.0 at 25^{o}C (EPA, 2005). Atrazine presents water solubility 28 mg/L (20^{o}C) and partition coefficient 2.3404 [3]. Simazine presents water solubility 3.5 mg/L and log of partition coefficient 1.94^{-3} [4].

Figure 1 shows the chemical structures of triazine herbicides. All compounds are poorly soluble in water and soluble in lipids, as described above. For this reason, these compounds readily cross biological membranes, being easily absorbed by the intestinal tract and the skin and mucous membranes [5].



Substitution at ring position:

Atrazine: **2**: -Cl; **4**: -C_2H_5; **6**: -$CH(CH_3)_2$

Simazine: 2: -Cl; **4**: -C_2H_5; **6**: -C_2H_5

Ametryn: 2: -SCH_3; **4**: -C_2H_5; **6**: -$CH(CH_3)_2$

Figure 1. Chemical structure of the selected s-triazines.

For animals and humans, the main access rout of the triazine herbicides is the oral absorption [6]. Biotransformation starts in the gut and these xenobiotics are partially metabolized and the triazines and its metabolites are found in plasma. McMullin et al. [5] studied the plasma concentrations of atrazine and its metabolites after oral gavage and observed different retention times and concentrations in the plasma for each metabolite. Atrazine (150 mg/body weight) presented a 25 hours concentration peak with maximum concentration at ~3 hours (~7 µmol/L). Direct metabolites monodealkylated, ETHYL (2-

chloro-4ethyl-amino-6amino-1,3,5-triazine) presented a 20 hours peak concentration with maximum concentration at ~3 hours (2.8 µmol/L), and ISOPROPYL (2-chloro-4-amino-6-isoppropylamino-1,3,5 triazine) presented a 18 h concentration peak with maximum concentration at ~3 hours (0.8 µmol/L). The subsequent metabolism of these dealkylated intermediates produces the di-dealkylated metabolite, diaminochlorotriazine (DACT) [7] that presented a 70 hours peak in the plasma with maximal concentration at -18 hours. 64% of oral administered atrazine was systemically metabolized by the cytochrome P450 family in the intestine to the monodealkylated metabolites [5].

When a xenobiotic enters the cell, is induced a biotransformation process to avoid damage and to facilitate its excretion. In mammals, the biotransformation processes occurs mainly in the liver, but all cells can do this. Neurons, skin and kidney cells, among others, have active biotransformation systems [8]. As described above, the biotransformation of triazines that occurs in the absorptive cells of the intestine is very important mainly for atrazine.

In the cells the first line of biotransformation is the microsomal, located at smooth endoplasmic reticulum, performed by cytochrome P450 system superfamily (CYP). These systems catalyze oxidation of substrates by O_2. One atom enters the substrate and the other forms H_2O. A reducing agent is required in liver is the NADPH [8]. The overall reaction is described by the reaction below where SH represents the substrate and RH_2 the reducing agent:

$$SH + O_2 + RH_2 \longrightarrow SOH + R + H_2O$$

Overload of CYP activity induces leaking of electrons leading to O_2 univalent reduction producing superoxide radical ($O_2^{-\cdot}$). This radical can start a series of cascade of oxidative reactions mediated by reactive species which can lead to cellular damage. To resist oxidative attack, cells and biological fluids possess two antioxidant defense systems. The first is the enzymatic antioxidant system constituted, among others, by the activity of the enzymes superoxide dismutase (SOD), catalase and the enzymes of glutathione redox system. Reduced thiol protein groups, metabolites like urate, reduced glutathione (GSH) and birubin, and several dietary products (ascorbate, tocopherols, carotenoids, flavonoids etc.) are examples of second enzymatic system constituents [9].

Glutathione redox system deserves further explanation, because its importance to the cellular redox status maintenance. Changes in the cellular redox state, even very small ones are related to processes of cell damage and also the adaptive processes. Reduced glutathione (GSH) is a tripeptide synthesized by the enzyme system controlled by glutathione sintethase, mainly in liver [8, 10]. Cells can import GSH using the χ-glutamil acil transferase (χ-GT) transporter system of membranes. GSH is the substrate of the enzyme glutathione peroxidase (GPX) used to detoxify H_2O_2 and organic hidroperoxydes, oxidizing GSH to GSSG (oxidized glutathione). To maintain the high GSH/GSSG ratio (100:1), GSSG is reduced to 2GSH by the enzyme glutathione reductase (GR) that uses the reducing power of NADPH. Disturbs in the pentose phosphate pathway (that produces most of NADPH) induces lipid peroxidation in the erythrocyte membrane [10].

In the liver, the dominant metabolic pathway of atrazine and simazine in mammals is N-monodealkylation and isopropylhydroxilation, by the cytochrome P450 metabolic system.

Also reported is that atrazine and simazine are metabolized to N-deethylated, N-deisopropylated and isopropylhydroxylated products by the P450 system in rats [7].

Other important reactions of biotransformation are the addition to xenobiotics of GSH or glycolic acid to increase water solubility to facilitate excretion. Were found in blood and urine adults of chlorinated metabolites of chlorotriazines (atrazine and simazine) conjugated with GSH [5]. Literature presents increase of activity of the enzyme glutathione-s-transferase (GST), that add GSH to xenobiotics, in the liver in the presence of atrazine, and diminishment of antioxidant enzimes activity in peripheral susceptive tissues as testicular interstitial cells [11] leading to an oxidative situation.

Both biotransformation systems induced by the herbicides can induce or increase an oxidative stress situation. As described above the CYP activity overload increases oxidative species production, and the complexation reactions decreases the GSH/GSSG ratio because the use of GSH. During oxidative stress, the reactive species and/or it derivatives can attack lipids, proteins and nucleic acid molecules causing damages that cause metabolic alterations which may lead, in extreme conditions, to cellular death. Oxidative stress has been associated with the onset or progression of many diseases such atherosclerosis, cancer, psoriasis, Alzheimer, hypertension and heart and liver diseases and with important physiological processes such aging and physical exercise training [8, 12].

Other authors demonstrated triazine herbicide established oxidative stress in brain and kidney [13], brain, muscle and liver [14], liver [15, 16] erythrocytes [17, 18] of many animal models.

TRIAZINES AND CELLULAR DAMAGE

Catabolism of atrazine and other chlorotriazines, including simazine and propazine, occurs in animals by dealkylation, dechlorination, and conjugation [19]. After these processes, the metabolites will be capable of interact with cells through biomolecules affecting cellular, tissue and organisms metabolism. Baker et al. [20] demonstrated that triazines negatively affected amphibian survival and highlighted that understanding how different chemical classes of pesticides and fertilizers interact with amphibian populations can lead to new management practices and regulations. Cellular markers applied *in vivo* or *in vitro* can lead valuable information in many different levels of investigation using varied animals groups. Besides, a lot of research resulting from atrazine effects evaluations [21] and ametryn and simazyne effects are little known.

Pathological changes are powerful indicators of exposure to environmental stressors. However, studies concerning the effects of pesticide exposure on tissue histopathology are scarce. Common carp sub-chronically exposed to atrazine contamination were negatively affected at the tissue level. Atrazine exposure caused alterations to the brain and kidney structure of the common carp, as evidenced by the degeneration of Purkinje cells in the brain and hydropic degeneration of the kidney. Different degrees of granule cell loss in the hippocampus, reduction of Nissl bodies, degeneration of Purkinje cells, neuropil loss were observed. The kidney of common carp displaying different degrees of cloudy swelling of epithelial cells of renal tubules, necrosis in the tubular epithelium, contraction of the

glomerulus and expansion of Bowman's space. So far, SOD, GSH-Px and CAT activities in the brain and kidney decreased after atrazine exposure [13].

Subchronic exposure of *Prochilodus lineatus* to 2, 10 and 25 µg/L of atrazine changes mechanisms of osmo and ionic regulation but does not result in significant changes in the Na^+/Cl^- ratio. Morphological responses exhibited by the gill cells explain, at least in part, the mechanisms of ion uptake maintenance and compensate for the possible effects of atrazine on the gills. Increase in plasma ion levels was observed that may have a cumulative effect leading to the significant increase observed in osmolality. These changes may be related to water shunts between plasma and interstitial fluid, suggesting altered water homeostasis and/or the changes in the concentration of metabolites in the blood [22].

Liu et al. [23] provided a partially understanding of the cytotoxic effects on fish cells caused by atrazine. These authors demonstrated that atrazine exhibited cytotoxic effects in cultured carp cells ZC7901 because of the induction of apoptosis.

Proteomic analysis was realized in adult female zebrafish (*Danio rerio*) liver exposed to atrazine. Several upregulated proteins were identified as 4-Hydroxyphenylpyruvate dioxygenase (HPPD) and heat shock protein (HSP) 10, which are responsible for stress response in zebrafish. In contrast, the main downregulated spots were identified as class III alcohol dehydrogenase, fatty acid-binding protein 7, and coatomer protein complex subunit zeta 1. These dates can aid to the development of new biomarkers that will be specific for pesticides allowing us to better understand the underlying mechanisms of atrazine-induced toxicity [24].

Atrazine sub-chronic exposure to 400 mg/kg/day concentration was able to induce hepatic rat oxidative stress, which resulted in higher levels of catalase and HSP90 but did not alter HSP70 levels or the levels of antioxidant enzymes (SOD and GST). Was also induced lipid peroxidation (LPO), hepatic degeneration accompanied by hepatocyte death, and the formation of micronuclei, confirming the cytotoxic and mutagenic potential of this herbicide [16].

Erythrocytes are highly susceptible to oxidative damage due to the presence of heme iron, polyunsaturated fatty acids (PUFA) and oxygen, which may initiate the reactions that induce oxidative changes in these cells. Some of the major alterations observed after atrazine treatment in rat erythrocytes were mild to moderate distortion in shape, significant ruptured membranes, echinocyte formation and central or peripheral protuberances. Authors suggested that these deformations might have occurred due to the underlying deformation of cytoskeleton in response to oxidative stress that induced an increase in lipid peroxidation (LPO) and changes in lipid composition of the membranes [17].

Similarly, Bhatti et al. [18] showed induction of erythrocyte LPO, changes the membrane content, and activities of antioxidant enzymes, suggesting that ROS may be involved in the toxic effects of atrazine. The changes in the cholesterol and phospholipid contents may be responsible for the changes in the activities of the membrane-bound acetylcholinesterase (AChE).

MCF-7 cells (derived from human breast cancer) were exposed to environmentally relevant concentrations of atrazine and were observed that this endocrine disrupting affected the proteomic level in these cells. Proteins belonged to various cellular compartments (nucleus, cytosol, membrane) and predominantly involved in transcription processes, stress regulation and structural components were underexpressed during the atrazine treatment indicating that atrazine treatment seems to decrease the activity of the cells [25].

Although the precise mechanism of action remains to be elucidated at certain tissue sites, studies have demonstrated that atrazine adversely affects the endocrine system and reproductive tissues in the rat. In a review presented by Sifakis et al. [26], pesticides can interfere with the hypothalamopituitary axis that regulates, through the production of the gonadotrophins FSH and LH, the function of Sertoli and Leydig cells, impairing spermatogenesis and steroidogenesis. Atrazine seems to have estrogenic and antiandrogenic properties and was suggested to reduce testicular testosterone and impair semen quality in male rats.

Sertoli-Germ Cells obtained of immature Wistar rats were submitted to concentrations of atrazine that corresponds to atrazine testicular tissue levels *in vivo* at the effect dose of 50 mg/kg/day. Atrazine-induced oxidative stress and decreased cell viability and increase of LPO and ROS production after a shorter period of culture than cell death, suggesting that atrazine-induced oxidative stress is the mechanism for reduction in cell viability. In addition, the increase in the activities for GPx and GR and their mRNA levels clearly show that the observed changes in enzyme activities are due to upregulated transcription of these genes. Increase in the antioxidant gene expression seems to be an adaptive response to oxidative stress, especially when atrazine has been reported to induce lipid peroxidation in several models [27].

According Pogrmic et al. [28], exposure to atrazine affects Leydig cell steroidogenesis via the inhibition of steroidogenesis gene expression, which is accompanied by decreased androgenesis. An interesting study developed by Pogrmic-Majkic's research group [29] to investigate direct effects of atrazine on rats Leydig cell steroidogenesis and the possible mechanisms of actions. They showed stimulatory effects of atrazine on cAMP accumulation and androgen production during the first 3 days of *in vivo* treatment (200 mg/kg body weight, by gavage) followed by a decline during further treatment, what indicates that atrazine has a transient stimulatory action on cAMP signaling pathway in Leydig cells and that further study would clarify duration of treatment when stimulatory atrazine action turns to inhibition.

Date yet no published obtained by our research group showed that an another triazine, ametryn, when orally administered for a chronic period was also capable to reduce sperm and cell Leydig number probably in consequence of oxidative stress induction, causing an impact on rat reproductive capacity. It is probably that ametryn have a similar atrazine mechanism of action.

TRIAZINE EFFECTS ON GENETIC MATERIAL

Excessive generation of intracellular ROS submits the organism to a process of oxidative stress and DNA damage [30, 31], leading to DNA base changes, single and double strand DNA breakage (SSB and DSB), and lesions in apurinic or apyrimidinic sites (AP sites) [32].

Oliveira-Brett and Silva [33] proposed that besides ROS induction, triazine herbicides can also interact with DNA directly through insertion mechanisms and formation of adducts between purine bases (Adenine and Guanine).

The agents that interact with DNA or its cellular components (spindle fibers) and enzymes (topoisomerase) are known to be genotoxic. The term genotoxicity refers to the nuclear changes caused by strand DNA breakage, abnormal DNA synthesis, and exchanges

between sister chromatids. The genotoxic effects may be transient and susceptible to repairs, while mutagenic effects feature permanent changes in the content or structure of the genetic material of an organism [34].

There are different biological assays for determining the genotoxicity and mutagenicity of triazine herbicides in vertebrate organisms. However, the main assays are the comet and micronucleus assay (Mn), which can be performed *in vitro* or *in vivo*. The comet assay, also known as SCGE (single-cell gel electrophoresis) consists on DNA migration over a blade covered with agarose under electrophoretic conditions. The material is observed with a fluorescence microscope, and damaged cells show the appearance of a comet, including a head (nuclear region) and a tail containing the fragments of DNA. This assay is fast, inexpensive, and sensitive enough to assess the primary damages in the genetic material, and can be applied in different tissues and specific cells [35- 38].

Among the available assays for evaluating the xenobiotic mutagenicity, the Micronucleus assay (Mn) in bone marrow and peripheral blood of rats and mice is one of the most accepted tests in the field of genetic toxicology because it allows the systematic evaluation of the substance of interest, and the metabolic activation can be a differential parameter *in vivo* and *in vitro* studies [39, 40]. As a general rule, the formation of micronuclei is the result of lagging acentric fragments to be deleted from the nucleus during mitosis [41]. Micronuclei appear in daughter cells due to, unrepaired or incorrectly repaired damages, induced in the parental cells that are formed during mitosis (telophase) or meiosis [42].

Information on the genotoxic potential of triazine herbicides is still contradictory. Several test organisms have been exposed to different concentrations and during different exposure periods for elucidation of the biological responses caused by these compounds on the genetic material.

Fish represents a standard model for the study of aquatic ecosystems because they are exposed directly to chemicals and mutagens from agricultural production runoff, or indirectly through the ecosystem food chain [43].

Cavas [44] evaluated the genotoxicity of the Gesaprim® herbicide, which has atrazine is the active principle, in peripheral blood erythrocytes of the fish *Carassius auratus* L., 1758, (Pisces: Cyprinidae) using the Mn and comet assays. The fishes were exposed to concentrations of 5, 10 and 15 mg/L of Gesaprim® for a period of 2, 4 and 6 days. An increase in the frequency of micronuclei and DNA damage were observed in all treatments, showing a genotoxic potential of this herbicide. Concordant results were found by Campos Ventura et al. [45] in peripheral blood erythrocytes of the fish *Oreochromis niloticus*, treated for 72 h with concentrations of 6.5, 12.5 and 25 mg/L of atrazine. Genotoxic effects were also observed by Nwani et al. [46] in erythrocytes and gill cells of the fish *Channa punctatusi* after concentrations of 8.4 mg/L on the seventh day of exposure (Figure 2). These results support that triazine shows a genotoxic potential on aquatic organisms.

Genotoxic effects of triazine herbicides have been found *in vivo* tests in rodents, suggesting a possible genotoxic potential of these herbicides in mammals. Singh et al. [17] evaluated the atrazine genotoxicity in male Wistar rats. The animals were treated with 300mg/kg during a period of 7, 14 and 21 days, and shown a significant increase ($p < 0.001$) in the tail length of comets and a significant increase in the frequency of micronuclei in blood and liver cells.

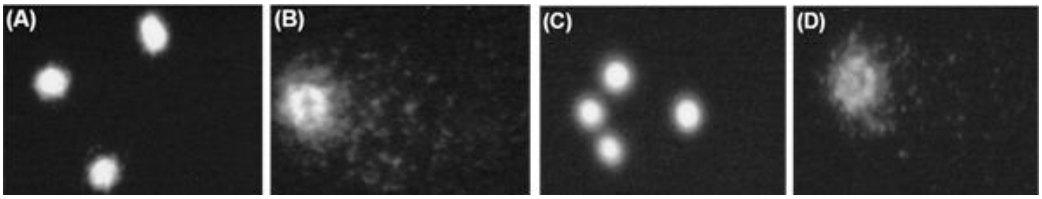


Figure 2. (A) Control, (B) atrazine exposed gill cells; (C) Control, and (D) atrazine exposed erytrocyte cells (adaptated of Nwani et al. [46]).

Campos-Pereira et al. [16] evaluated the bone marrow of the same model treated with 400 mg/kg body weight of atrazine for a period of 14 days, and observed an increase in the frequency of micronucleated polychromatic erythrocytes. However, Kligerman et al. [47], evaluated the genotoxicity of three triazine herbicides in the mouse bone marrow in different concentrations: Atrazine (125, 250 and 500 mg/Kg), simazine (2000 mg/kg) and cyamazine (100, 200, 300 and 400 mg/kg). They did not observe increases in micronuclei. Their results suggest that the triazine herbicides not have genotoxic potential in bone marrow Mn under the tested conditions. Some authors suggest that the toxic potential of some triazine herbicides can be reduced because of a conjugation with reduced glutathione (GSH) caused by the molecular structure of these herbicides and by the glutathione-S-transferase (GST) action [15].

Genotoxicity of the simazine herbicide was assessed through different techniques. The results were concordant *in vivo* and *in vitro* studies with no changes in the measured genetic parameters. The Mn in bone marrow and peripheral blood of rodents did not show significant changes, as well as tests of sister chromatid exchanges in Chinese hamster cells [48]. The information available in the literature suggests that simazine herbicide do not show genotoxic or mutagenic potential.

Ametryn herbicide has been poorly studied for biological effects in living organisms, but the genotoxic potential was verified by Maya-Flores et al. [49] and Calderon-Segura et al. [55]. Both groups studied the genotoxicity of this compound with direct application in cultured human lymphocytes, and with the application of extracts from the roots of *Vicia faba* treated with different concentrations of ametryn. The direct treatment performed with the herbicide did not induce sister chromatid exchanges, and no change in the comet assay was observed. However, genotoxic effects were observed in cells treated with the extracts of the roots of *V. faba* exposed to ametryn. These results indicate that this herbicide goes through a process of biological activation and produces active metabolites when metabolized by the plant. These metabolites are able to induce genetic damage in human lymphocytes cultures. The genotoxic effects using different assays systems are listed in table 1.

Triazine herbicides were extensively evaluated in various genotoxicity assays and different doses and concentrations were tested under different conditions. However, the results on its mutagenic potential are inconclusive [56].

Experimental studies have associated triazine herbicides to the development of cancer in rats. Sprague-Dawley rats developed mammary adenocarcinoma cells when exposed to atrazine, suggesting this herbicide could possibly be classified as a probable human carcinogen. However, more elaborate studies concluded that the mechanisms involved in tumor formation had a hormonal origin and were not associated with DNA damage. These

results supported the classification of atrazine as "not likely to be carcinogenic in humans" by the United States Environmental Protection Agency (U.S. EPA) [57].

Table 1. Summary of positive results about genotoxicity of triazines

Test Organisms	DNA effect	Triazine	Dose	References
Japanese quail	Increased comet assay	Atrazine	500 mg/kg	Hussain et al., 2011 [50]
Peripheral blood leukocytes of mice	Increased comet assay	Atrazine Cyanazine	500 mg/kg 200/300 mg/kg	Tennant et al., 2001 [51]
Workers occupationally exposed	Increased comet assay Micronucleus test	Mixture of pesticides (atrazine, cyanazine)	——	Garaj-Vrhovac, Zeljezic, 2002 [52]
Multiple mouse tissues	Increased comet assay	Gesaprim® (atrazine)	540 mg/kg	Zeljezic et al., 2004 [53]
Human lymphocytes *(in vitro)*	Increased comet assay	Gesaprim® (atrazine)	0.47 e 4.7 µg/mL	Zeljezic et al., 2006 [54]
Human lymphocytes *(in vitro)*	Increased comet assay	Ametryn	50-500 mg/L	Calderon-Segura et al., 2007 [55]
Oreochromis niloticus (Fish)	Increased comet assay Micronucleus test	Atrazine	6.5, 12.5 and 25 µg/L	Campos Ventura et al., 2008 [45]
Wistar rats (liver and blood cells)	Increased comet assay Micronucleus test	Atrazine	300 mg/kg	Singh et al., 2008 [17]
Carassius auratus (Fish)	Increased comet assay Micronucleus test	Gesaprim® (atrazine)	5, 10 e 15 µg/L	Cavas, 2011 [44]
Channa punctatus (Fish)	Increased comet assay Micronucleus test	Rasayanzine	8.4 mg/L	Nwani et al., 2011[46]
Prochilodus lineatus (Fish)	Increased comet assay	Atrazine	10 µg/L	Santos and Martinez, 2012 [15]
Wistar Rats (bone marrow)	Micronucleus test	Atrazine	400 mg/kg	Campos-Pereira et al., 2012 [16]

Studies conducted with workers in contact with triazines show that different types of cancers are reported, however, none of the results are statistically significant. The epidemiological studies do not provide convincing scientific proof of a causal relationship between triazine herbicides and cancer in humans [57-60].

Another interesting aspect is available recent evidence that support the concept that epigenetics holds substantial potential for furthering our understanding of the molecular mechanisms of pesticides health effects, as well as for predicting health-related risks due to conditions of environmental exposure and individual susceptibility. Epigenetics effects are heritable changes in gene expression that occur without a change in the DNA sequence. Although no information about epigenetic effects of triazines, global DNA methylation levels have been reported to be inversely associated with blood levels of persistent organic pollutants (POPs), xenobiotics that accumulate in adipose tissue. So far, some pesticides that belong to the environmental endocrine disruptors (EDs) family, synthetic chemicals that resemble natural hormones, are also known to cause epigenetic perturbations [61].

TOXICITY AND CELL DEATH

A variety of environmental contaminants can induce apoptosis, necrosis, or autophagy, depending on both cell type and dose. Then, it is now apparent that multiple cell death programs can be activated during toxicity. It seems more likely that several death executing routines may be activated concomitantly within injured cells and that one or the other becomes predominant, depending on the stimulus and the metabolic state of the tissue. Although the predominance of one or the other death executing mechanism may be dictated by factors as different as energy requirement, signaling molecules or the intensity of a given insult, in many instances, the differentiation program within a given tissue dictates the way to die [62,63].

It is evident that chemical toxicity might be associated with multiple modes of cell death. There is the coexistence of different cell death modalities in pathological settings that involves an intricate cross talk of cellular signaling pathways. Depending on the type of lethal agent, the cell death process can be initiated in different intracellular compartments, and cross talk between these compartments appears essential for all cell death modalities. Importantly, depending on the nature and severity of the stimulus, and on the cell type, the hierarchy of interorganelle cross talk might result in different cell death modalities. Moreover, in some cases, suppression of the function of a particular intracellular compartment might switch one mode of cell death to another [63].

In this scenario, the classification of the cell death in tissues and organs of animals exposed to environmental chemical compounds become very difficult. Conversely, the presence or the absence of cell death in the histophatological analysis is an indicative of the level of stress triggers by herbicides in exposed animals and its detection is an important tool in ecotoxicological studies. On the basis of histopatological changes, such as alteration of tubular system of caudal kidney, the values of LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) for simazine were estimated in different developmental stages of common carp (*Cyprinus carpio*) [64].

Triazine herbicides can induce immunotoxicity. Atrazine, for example, is capable of inducing splenocytic apoptosis mediated by the Fas/FasL pathway in mice, which could be the potential mechanism underlying the immunotoxicity of atrazine [65]. Male mice which had been prenatally/lactationally exposed to atrazine had an increase in both T cell proliferation and cytolytic activity [66]. Atrazine-induced immunotoxicity is an example of

cell death induced by extracellular stress signals that are sensed and propagated by specific transmembrane receptors. According to review performed by Galluzzi et al. [67], this kind of cell death is termed 'extrinsic apoptosis'.

Extrinsic apoptosis can be initiated by the binding of lethal ligands, such as FAS/CD95 ligand (FASL/CD95L), tumor necrosis factor α (TNFα) and TNF (ligand) superfamily, TNF-related apoptosis inducing ligand (TRAIL), to various death receptors (i.e., FAS/CD95, TNFR1 and TRAILR1–2) [67,68].

The first evidence that atrazine was able to induce apoptosis in fish cells was performed by Liu et al. [23]. In this study, the authors indicated the existence of a novel cytotoxic mechanism caused by atrazine that may improve our understanding of the complex relationship between contaminants and aquatic organisms. Apoptosis induced by atrazine was dose- and time-dependent and was involved in mitochondrial membrane potential (DeltaPsi(m)) disruption, elevation in intracellular $Ca^{2+,}$ generation of reactive oxygen species, and intracellular ATP depletion. According to review performed by Galluzzi et al. [67], this kind of cell death is termed 'intrinsic apoptosis'.

Intrinsic apoptosis can be triggered by a variety of intracellular stress conditions, including DNA damage, oxidative stress, cytosolic Ca^{2+} overload, mild excitotoxicity (related to glutamate receptor overstimulation in the nervous system), accumulation of unfolded proteins in the endoplasmic reticulum (ER) and many others. Although the signaling cascades that trigger intrinsic apoptosis are highly heterogeneous, including exogenous chemical compounds such as toxicants, as far as the initiating stimuli are concerned, they are all wired to a mitochondrion-centered control mechanism. Frequently, along with the propagation of the pro-apoptotic signaling cascade, anti-apoptotic mechanisms are also engaged, in an attempt to allow cells to cope with stress. In this scenario, both pro- and anti-apoptotic signals converge at mitochondrial membranes, which become permeabilized when the former predominate over the latter [69]. Thus, intrinsic apoptosis results from a bioenergetic and metabolic catastrophe coupled to multiple active executioner mechanisms.

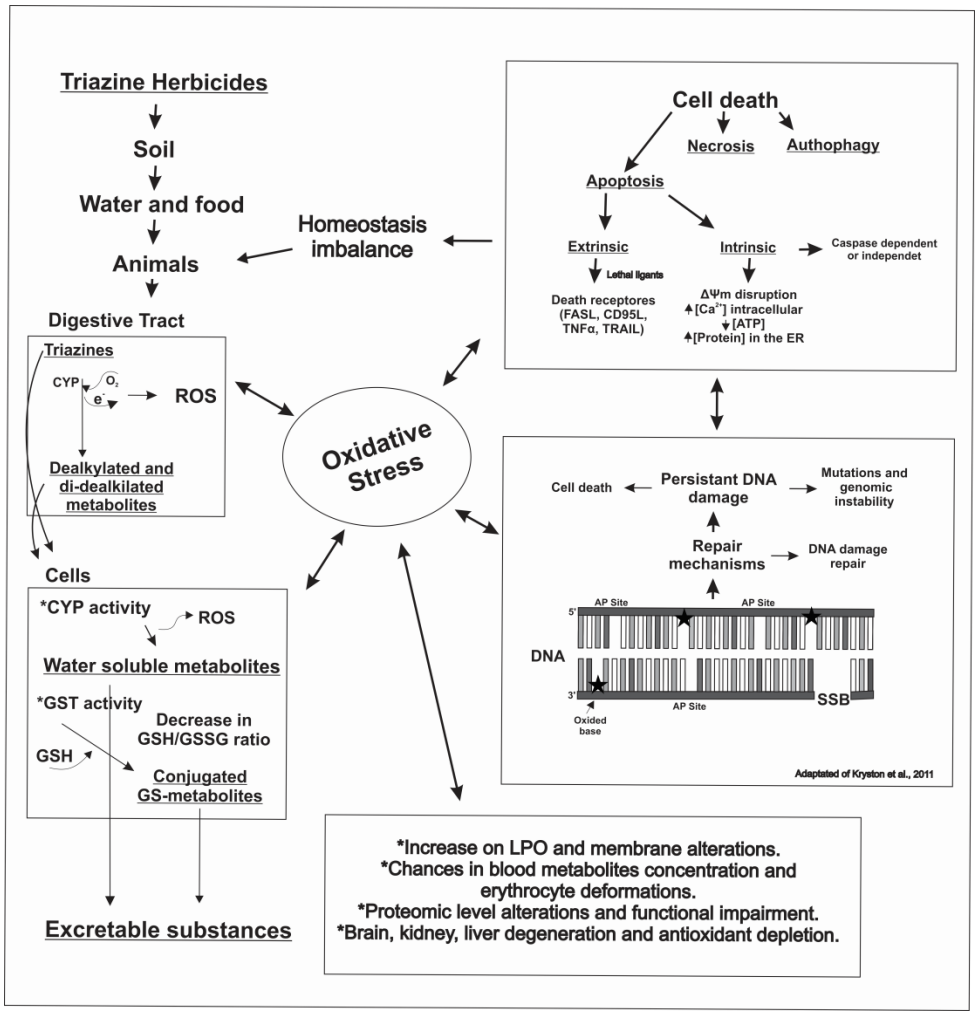
Intrinsic apoptosis can be caspase-dependent and caspase-independent. An example of caspase-dependent apoptosis was described by Zaya et al. [70] that performed a study regarding the effects of atrazine on developing *Xenopus laevis* tadpoles. Livers from 400 μg/L exposed tadpoles had higher numbers of activated caspase-3 immunopositive cells suggesting increased rates of apoptosis. It is important to highlight that this kind of apoptosis was detected in tadpoles exposed to low levels of atrazine that are potentially found in puddles, vernal ponds and runoff soon after application (200 and 400 μg/L).

Conversely, caspase activation seems to have a prominent role in a limited number of instances of stress-induced intrinsic apoptosis in vitro, as demonstrated by the fact that – in contrast to extrinsic apoptosis – chemical and/or genetic inhibition of caspases rarely, if ever, confers long-term cytoprotective effects and truly prevents cell death. In this context, caspase inhibition only delays the execution of cell death, which eventually can even exhibit morphological features of necrosis [71-73].

In spite of the recent advances in our understanding of cell death mechanisms and associated signaling networks, much work remains to be done before we can fully appreciate the toxicological significance of these findings. Although it is clear that the activation of cell death pathways is responsible for acute toxicity of many chemical toxicants, their potential involvement in subacute or chronic toxicity caused by long-term exposure to other drugs or environmental pollutants remains to be investigated.

CONCLUSION

The major triazine effects on vertebrates are summarized at the figure below.



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